PUBLIC HEALTH REPORT

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Laboratory Diagnostic Services For Rubella in Public Health Laboratories in California

THE STATE DEPARTMENT OF PUBLIC HEALTH has received a number of inquiries from physicians concerning laboratory tests for diagnosis of rubella. The department's Viral and Rickettsial Disease Laboratory has offered such tests since early in 1965. Intensive development and evaluation studies have resulted in significant improvements, both in virus isolation and serologic methods, which greatly broaden the practical applications of rubella laboratory tests in clinical practice. Thus, the State Virus Laboratory now offers tests:

- To confirm or rule out rubella in patients with exanthem or other suspicious clinical signs of rubella,
- To establish the diagnosis of congenital rubella virus infection in infants,
- To determine the immunity status of pregnant women exposed to rubella during pregnancy.

Tests for immunity are also provided for nurses and other medical personnel subject to high risk of exposure.

Other important uses of rubella tests include routine testing of women for immunity status at time of prenatal examination or before they become pregnant. With the release of attenuated live rubella virus vaccines expected shortly, the need for immunity tests will increase since tests for presence of rubella antibodies in women of childbearing age will offer a means of identifying those who are susceptible and may benefit from immunization. However, because of the overwhelming volume, the State Virus Laboratory cannot offer routine prenatal or pre-vaccination tests. Moreover, this need can be met most effectively through development of local laboratory resources.

The laboratory resources needed to carry out testing on so large a scale are not available in California. To provide such laboratory assistance to individual physicians and community health facilities will require that additional laboratories in various localities in California develop competence in performing tests for rubella.

To this end the State Health Department's Virus Laboratory has assisted a number of local public health department laboratories to acquire experience in rubella test methods. Several now offer this service locally and others are in the process of training personnel. A few clinical laboratories in California now offer serologic tests for rubella. Laboratories which have not had previous experience in the field of viral serology, however, face serious difficulties in undertaking tests for rubella until specialized professional training and experience with the tests have been acquired.

A clear warning of these difficulties was recently issued by the Medical Laboratory Services Advisory Committee of the U.S. Public Health Service as follows*:

"Serologic Testing for Rubella— A Warning"

"Serologic tests for rubella are primarily used to determine: (1) the immune status of individuals in a given population; (2) the immune status of pregnant women who have been exposed to rubella; and (3) the etiology of cases of exanthematous disease. In the first instance, results of tests are used for epidemiological and immunization planning purposes; in the second and third instances, results are used to provide information for making medical management decisions in situations of some urgency.

"At the present time the hemagglutination inhibition (HI) test is the technique most widely used for measuring rubella antibodies. This test is a complex procedure which must be performed by well trained, experienced individuals. In addition, a thorough knowledge of the immune response is essential for the proper interpretation of test results. Because of actions which may be taken on the basis of laboratory results, the need for accuracy is great, and certain problems associated with the HI test must be recognized.

"The HI test for rubella is not a standardized

Reprint requests to: Viral and Rickettsial Disease Laboratory, State Department of Public Health, 2151 Berkeley Way, Berkeley 94704.

*Quoted from the U.S. Public Health Service's weekly report "Morbidity and Mortality," Vol. 18, p. 126, 12 April 1969.

technique, and several modifications of the basic procedure are in use. Methods for removing nonspecific inhibitors in serum specimens may not be completely effective, or they may remove specific antibody, leading to false positive or false negative results. Reagents obtained from different sources are not uniform in quality or in suitability for all modifications of the HI test. Since the products from each manufacturer are for use in a specific HI procedure, intermixing reagents from different sources can lead to problems in test performance. Further, the wide variability of erythrocyte suspensions has considerable bearing on the sensitivity of the test. Because of the lack of uniformity in testing procedures and reagents, interpreting laboratory results is a sophisticated undertaking, and, of necessity, may vary from one laboratory to another.

"In view of the problems associated with this serologic procedure, HI tests for rubella should not be attempted in a laboratory carrying out the tests on an infrequent basis. Such a laboratory cannot maintain the necessary skills and controls, and, in urgent cases involving therapeutic abortion, pressures may lead to failure to repeat tests or to perform more difficult supplemental tests, such as complement fixation, fluorescent antibody, and serum neutralization tests, or IgM determinations which may be necessary for accurate interpretation.

"The laboratory asked to carry out HI tests for rubella only infrequently or to perform supplemental tests for which it is not qualified should refer diagnostic materials to a state health department or other competent reference laboratory."

Standardizing Methods and Training Personnel

The Virus Laboratory of the California Department of Public Health is collaborating with the National Communicable Disease Center (NCDC) to assess various modifications of the HI antibody test procedure to assist in establishing recommendations for an optimal standard method. The NCDC and state laboratories also perform tests with commercially distributed rubella test "kits" and reagents to evaluate performance. Difficulties have occurred in performing the test with some of the commercial products.

The laboratory Proficiency Testing Program recently initiated by NCDC provides that each participating State laboratory test identical specimens and compare their results with those of selected reference laboratories. The state laboratories can thus recognize deficiencies in the quality of their results and seek out sources of error.

In California, local public health laboratories that perform tests for rubella may send representative specimens, together with their own results, to the State Health Department Virus Laboratory for confirmation. If significant discrepancies occur, consultation and assistance are available to define and correct the error.

The State Virus Laboratory is conducting workshops on the rubella HI test to assist in training of local public health laboratory personnel. Instructions are given on the recommended procedure, sources of satisfactory reagents and problems in interpretation of results. To be eligible for these workshops, applicants must be graduate microbiologists with at least six months' experience in a public health laboratory, or equivalent. In addition, the virology program for microbiologist trainees at the State Virus Laboratory includes thorough instruction in rubella HI tests. Representatives from 25 local public health laboratories have completed this training. The experience gained by these laboratories will offer a local resource to help other local laboratories become proficient in the rubella HI test.

Clinical laboratories contemplating rubella HI tests should have an experienced microbiologist who has obtained specific training in this procedure.

Interpretation of Rubella Serologic Tests

In clinically apparent infections of children and adults rubella HI antibodies generally appear by three to five days after onset of symptoms and increase to a maximum titer by 14 to 21 days after onset. Antibodies persist thereafter for many years, perhaps for life, gradually declining in titer. There are pronounced individual variations in the maximum antibody reached and the duration of highly elevated titers. One patient at two weeks after onset may have the same antibody titer as another who had rubella two or three years ago. Thus, for serologic tests to be useful for supporting clinical judgements, especially those concerned with rubella, or exposure, in pregnancy, the time at which specimens are collected is of critical importance.

For confirmation of a suspected case of rubella, it is essential to demonstrate a significant rise in antibody titer between two serum specimens, the

first drawn promptly after the appearance of the rash or other clinical signs, and the second at 14 to 21 days. Only a few days' delay in obtaining the initial specimen may preclude judgement as to whether an antibody titer is related to the present illness or represents residual antibody from a past infection.

To determine if a patient is already immune at the time of exposure to a case of rubella, the serum must be obtained promptly, preferably within seven days, but no later than ten days, after the exposure. After ten days, it becomes increasingly uncertain whether any antibody demonstrated reflects existing immunity at the time of exposure or an infection subsequently acquired. When a single specimen obtained promptly after exposure shows the presence of antibody, the patient is considered to be immune and follow-up specimens are not usually needed.

When paired specimens are tested to demonstrate a change in antibody titer, they must be tested simultaneously, utilizing the same reagents and identical test conditions in order to minimize inherent variations in the test. Hence, the first specimen is held by the laboratory until the second is received and can be tested together with the first.

Specimens for Rubella Serologic Tests

In summary, the specimens for serologic tests should be collected as follows:

Type of specimen: 6 to 8 ml of whole clotted blood; no preservative.

1. For diagnosis of clinical case; submit two specimens.
   a. Acute-phase as soon as possible after onset (after five days may be too late to show diagnostic rise in antibody titer).
   b. Convalescent-phase, 14 to 21 days after onset.
2. For immunity status at time of exposure; submit one specimen as soon as possible after exposure; no later than seven to ten days.

Information to Accompany Specimens

For the laboratory to proceed intelligently and interpret test results a request form must accompany the specimen providing pertinent information on the clinical circumstances which have prompted the request as follows:

- Identification of patient: Name, age, sex.
- Date specimen collected.
- Reason for test; indicate clearly whether for:
  a. suspected clinical case
  b. exposure only; no clinical signs
  c. suspected congenital infection
  d. other circumstances; e.g., nurse, subject to exposure in clinic.
- If clinical case: give date of onset.
- If exposed only: give date(s) of exposure.
- Indicate if patient is pregnant: give date of last menstrual period or estimated week of gestation.
- If suspected congenital infection: give date of birth, signs of congenital rubella, maternal history of rubella.

Public Health Laboratories Performing Tests

As services are now available locally or soon will be in various areas, local laboratories or physicians wishing to obtain tests for rubella are advised to contact their local health department for information.

In those jurisdictions where services are not available, the Virus Laboratory of the State Health Department will perform tests for rubella upon submission of appropriate specimens and clinical information as outlined above. *The State Virus Laboratory, however, is unable to provide routine prenatal immunity testing, routine testing in anticipation of pregnancy, or administration of vaccine.*

Laboratories of the local health departments listed below are now or soon will be offering tests for rubella. In these areas, to avoid delays, requests for rubella tests should be submitted directly to the local laboratory and the local laboratory should be contacted regarding request forms, specimen containers, or instructions for submitting specimens:

Alameda Co. Health Dept.,
Oakland, 499 5th St.

Contra Costa Co. Health Dept.,
Martinez, 1111 Ward St. (P.O. Box 871)

Fresno Co. Health Dept.,
Fresno, 515 So. Cedar Ave.

Los Angeles Co. Health Dept.,
Los Angeles, 220 N. Broadway

Orange Co. Health Dept.,
Santa Ana, 645 No. Ross St. (P.O. Box 355)

San Diego Co. Health Dept.,
San Diego, 1600 Pacific Highway

Santa Clara Co. Health Dept.,
San Jose, 2220 Moorpark Ave.

Yolo Co. Health Dept.,
Woodland, 10 Cottonwood St.
(P.O. Box 1157)